EFFECT OF BIOLOGICAL NITRIFICATION INHIBITOR ON NITROGEN FIXATION AND EMISSION REDUCTION IN STRAW AND DAIRY MANURE COMPOSTING

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Abstract. The purpose of this study was to analyze the effect of biological nitrification inhibitor MHPP on nitrogen fixation and emission reduction for straw and dairy manure composting. During the period from May to September in China, the engineering large-scale composting was performed with dark box sampling was conducted to take composting gas sample which was studied gas chromatography. The result indicated that, in relation to the control, to which no MHPP extract solution was added, the addition of 0.2%, 0.4%, 0.6% and 0.8% MHPP extract solutions reduced the methane emission by 44.52%, 56.56%, 61.93%, 59.59%, the N_2O emission by 36.24%, 39.49%, 62.39 and 55.81%, and the NH_3 emission by 23.72%, 65.61%, 65.40% and 72.61%; after the addition of biological nitrification inhibitor, the composting process slowed down; the ammonium nitrogen content was high while the nitrate nitrogen content was low; all substances thoroughly decomposed in the end. Moreover, the final ammonium nitrogen content was higher in all cases when compared to the control. The results showed that the addition of 0.6% MHPP extract solution worked the best for nitrogen fixation and emission reduction and thus is appropriate to be used extensively in production activities.
Keywords: *crop residue composting, N_2O, NH_3, CH_4, biological nitrification inhibitor*

Introduction

With the increase of grain output in China, the straw resources grew constantly to more than a billion tons; furthermore, the mushrooming of livestock and poultry feeding industry in China produced a lot of waste. Both are considered environmental difficulties of rural areas and agriculture. As one of the effective approaches of fertilization against non-point source pollution in the planting and breeding industry, high-temperature aerobic composting has aroused wide concern all over the world, and turning into a research focus in the field of eco-friendliness and environmental protection.

High-temperature composting technology that may bring about non-point source pollution is widely recognized in agriculture, but researchers argued that the emission of some harmful gases and greenhouse gases, including NH_3, CH_4 and N_2O, may cause secondary pollution to a certain extent during high-temperature composting. During composting, the degradation and nitrification and denitrification of organic nitrogen produces a certain amount of NH_3 and N_2O; the emission of N-containing gas result in a nitrogen loss that range from 16% to 76%, which not only impairs the quality of compost, but may also lead to environmental pollution (Wu et al., 2012). Moreover, ammonia is not only an important factor affecting acid rain, but is also a key component

of odor substances in composting plants; the Emission Standard for Odor Pollutants (GB14554-93) issued by China in 1993 announces ammonia as the primary odor pollutant. According to the IPPC report 2014, the 100a greenhouse effect produced by N_2O and CH_4 was 25 and 298 times that of CO_2, respectively (IPCC, 2014). From this perspective, the emission of ammonia and greenhouse gases during composting not only adversely reduced the land use value, but also resulted in odor pollution, acid rain, greenhouse effect and other new ecological and environmental problems; in addition, they may harm human and animal health. Hence, along with the growing sophistication of composting technology, how to reduce nitrogen loss and greenhouse gas emission during composting has become a research focus.

Adding chemical additives like peat, calcium superphosphate, ferric chloride, zeolite and clay (Yang et al., 2005) is considered one of the effective ways to emission realize nitrogen fixation and emission reduction. Liu et al. (2015) found that adding 3% bamboo acetic acid to compost materials helped to reduce greenhouse gas emission and improve compost quality. Works of Ren et al. (2008) showed that the nitrogen fixation rate reached 73.1–97% with the addition of phosphate and magnesium hydroxide, etc. during composting; Jiang et al. (2018) found that NH_3 volatilization was significantly reduced during aerobic degradation of pig manure and corn straws by adding calcium phosphate and phosphoric acid.

Through the research on generation and regulation of odor in pig manure composting, Luo et al. (2012) found that the addition of calcium phosphate during pig manure composting not only reduced the compost pH and the concentration of volatile NH_3, but also facilitated the transformation of NH_4^+-N into other forms of nitrogen, thereby improving nitrogen retention. Lin et al. (2008), Weng et al. (2012) and Ren et al. (2009) found that the addition of calcium phosphate helped to reduce NH_3 emission during composting and thus retain nitrogen. Jiang et al. (2016) found in his study that the addition of nitrification inhibitor dicyandiamide (DCD) based on the use of acidic nitrogen fixer reduced the total greenhouse gas emission by 78% and NH_3 emission by 53%. The added nitrification inhibitors (NIs) are a generic term of a class of chemical substances that inhibit nitrification reaction by inhibiting the activity of nitrifying bacteria, thereby reducing nitrogen loss. DCD is widely used in Europe and America as a NI, but it is seldom used for composting. Li et al. (2008) and Ji et al. (2011) found that DCD reduced CH_4 and N_2O emission of rice and wheat by over 20%. Luo et al. (2013) and Jiang et al. (2016) also confirmed that the addition of DCD during composting of pig manure and straws remarkably reduced N_2O emission. Chen et al. (2017) tried to use calcium superphosphate with DCD for sludge composting and achieved remarkable effect: The nitrogen losses were reduced by 13%, and the greenhouse gas emissions by 74.8%.

Although nitrification inhibitors are significantly effective in reducing N_2O emission, they are not extensively used in production due to their high cost and toxicity; hence, some researchers shifted their attention to lower-cost biological nitrification inhibitors. Zakir et al. (2008) identified the MHPP (methyl 3-(4-hydroxyphenyl) propionate) as a biological nitrification inhibitor from sorghum root exudate to inhibit nitrification in soil. Wei et al. and Zhu et al. (2015) studied the molecular mechanism of MHPP, but there are few reports on the application of MHPP in composting. In this study, MHPP was used for composting and is compared with DCD, the most commonly used nitrification inhibitor, to systematically study the effect of this new nitrogen fixation and emission reduction conditioner on the rule of nitrogen gas and greenhouse gas emission during fermentation of straws and dairy manure; its purpose is to provide a theoretical

basis for the selection of nitrogen fixation and emission reduction conditioner for straw and dairy manure composting.

Materials and methods

Test materials

The wheat straws for the test were taken from the Fengyang Plantation of Anhui Science and Technology University and was smashed to 8-10 cm in size. The fresh dairy manure was taken from the cattle farm of Fucheng Town, Fengyang County, Anhui Province. The initial properties of straws and dung before the test are shown in *Table 1*. The 2nd and 4th row used for calculate C/N ratio. The calcium superphosphate was purchased from Bengbu Tianyi Biological Agents Co., Ltd., and its effective component was defined as P_2O_5 ($\geq 18\%$); the dicyandiamide was analytically pure.

Table 1. Physical and chemical characters of raw materials

Materials	Moisture content (%)	Total organic carbon mass fraction (g/kg)	pH	Total nitrogen (g/kg)	Total phosphorous (g/kg)	Total potassium (g/kg)	C/N
Dairy manure	62.62±0.55	398.76±0.34	7.85±0.08	24.86±0.12	30.32±0.32	11.17±0.18	16.04
Wheat Straw	12.65±0.23	489.23±0.38	7.21±0.03	8.92±0.13	3.28±0.41	79.34±0.29	54.82

Values in average ± standard deviation (n = 3)

Test design

This test was performed at the plantation test field of Anhui Science and Technology University in China between May and September 2017. It was performed on a cement floor, and the composting treatment design was as follows: Fresh weight of dairy manure: straw = 1.5:1, and the treatment details are shown in *Table 2*. the MHPP leaching liquor was added once every seven days; the calcium superphosphate was added only once, and "blank" indicates no calcium superphosphate was added; refer to *Table 2* for details. 7.5 t of dairy manure and 3t of straws were prepared; the straws were smashed into 8-10 cm in size and mixed with the dairy manure with spray water to a proportion of approx. 65%; the mixture was divided evenly into five piles (4 t per pile); the temperature was raised to above 65 °C, at which manual turning was conducted with the help of weed-grasping machine every 7days. The compost temperature was determined on a daily basis (9:00 am) during composting, and samples were taken d0, d5, d10, d15, d20, d25, d30, d35, d40 and d45, respectively. The composting was ceased when the germination rate of Chinese cabbage seeds reached over 90%.

Test items and methods

Samples of greenhouse gases (CH_4, N_2O, and CO_2) were taken with static dark boxes customized from Fenglu Building Materials Store in Xuanwu District, Nanjing; the sampling case covered with aluminum foil (PVC, L × W × H = 50 cm × 50 cm × 50 cm) was placed over the pile; we measure thecircumference of the pile when we took gas sample. We calculate area by circumference. The pile is protected from disturbance and

sealed with water; set it aside for 30 min before the sampling was started, and gas was collected with a 50 ml syringe with a three-phase valve every 10 min (3 cycles in total); the sample test was finished within 24 h; where the test was not finished within 24 h, the gas was collected with a 100 ml sampling bag. CH_4 and N_2O were analyzed with the gas chromatograph (Agilient 4890, the USA) at the same time, and the CH_4 and N_2O concentrations were determined respectively with FID (Flame Ionization Detector) and ECD (Electron Capture Detector). The ammonia sample was taken with dark box through gas collection and ventilation, and the gas was sampled with a sampling pump; 10 ml of sulfuric acid absorbent was put in the bubble sampler, where its flow rate was 1.2 L per minute; the sampling process lasted for 10 min. Determination was performed with Nessler's Reagent. Weed-grasping machine used HTZC-600 (Henan Hengtong Machinery Co., Ltd.)，sampling pump used E-Switch-MRO292-1.2L (Shanghai Shenyuan Scientific Instruments Co., Ltd.) (*Photo 1*).

Table 2. Addition proportion of materials in each compost treatment

Treatment	Raw materials (fresh weight)	Percentage of additives to raw materials mass/%	
		Calcium superphosphate	Nitrification inhibitor
CK	Dairy manure: Straws = 1.5:1	0%	0
T1	Dairy manure: Straws = 1.5:1	0%	1%DCD
T2	Dairy manure: Straws = 1.5:1	2%	1%DCD
T3	Dairy manure: Straws = 1.5:1	0%	1% MHPP
T4	Dairy manure: Straws = 1.5:1	2%	1% MHPP

Photo 1. The equipment we used and experimental picture

Results

Effect of addition of MHPP addition on the variation of compost temperature

Temperature is an important indicator indicating of whether the organic matters are degraded rapidly during composting of straws and dairy manure. The treatment-specific temperature fluctuations during composting for this test are shown in *Figure 1*. In each

treatment cycle, the mesophilic microorganisms degraded the soluble and easily degradable organic matters in straws and dairy manure at low temperature in the initial phase of composting, where a lot of heat was released, resulting in the quick rise of compost temperature within a short time; with the decrease of available organic matters, the activity intensity of microorganisms decreased progressively, and the compost temperature dropped gradually; in this process, the compost became thoroughly decomposed.
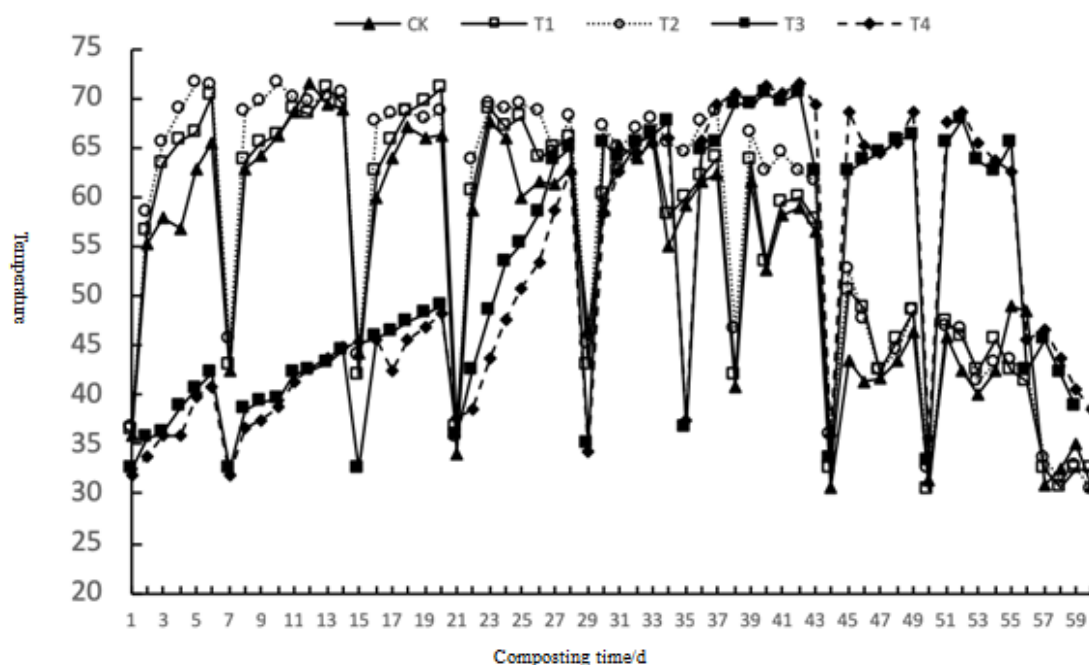

Figure 1. Change of temperature during composting period

Control and treatment 1 and 2 got into the high temperature period (> 50 °C) on d2 and went above 70 °C on d5; the heat-up effect was extremely obvious and higher than that of control; the high temperature lasted for approx. one month before the temperature starting to drop. The temperatures of Treatment 3 and Treatment 4 went up slowly at the beginning of composting, and reached 50 °C on d24; this may be because that the microbial activity was probably inhibited with the obvious inhibiting effect of MHPP; despite the slow rise, the high temperature lasted for approx. one month later. The high temperature period (> 50 °C) of each treatment lasted for about one month, which was much longer than 5 days for innocuous treatment according to the national regulations of China; this helps to kill pathogenic microorganisms and pathogenic eggs during composting, thus assuring the innocuous treatment of composting.

Effect of the addition of MHPP addition on emission of methane as a greenhouse gas from compost

CH_4 is a greenhouse gas produced by methane bacteria from the simple organic matters like methylamines, methyl alcohols, methyl acids and other carbon-containing compounds, as well as carbon dioxide and hydrogen in straws and dairy manure in pile. As shown in *Figure 2,* the methane emission peaks of all the four treatments occurred at

early stage of composting; the quick rise of temperature in early stage stimulated microorganisms to degrade organic matters efficiently, which consumed a lot of oxygen; as a result, the excessively low locally soluble oxygen content facilitated the production of high content of CH_4. As the composting progressed, the organic carbon available in straws and dairy manure was consumed progressively, and the microbial activity decreased, and the CH_4 emission rate went down gradually; in the end, the degradation of organic matters stopped, so CH_4 emission was hardly detected.
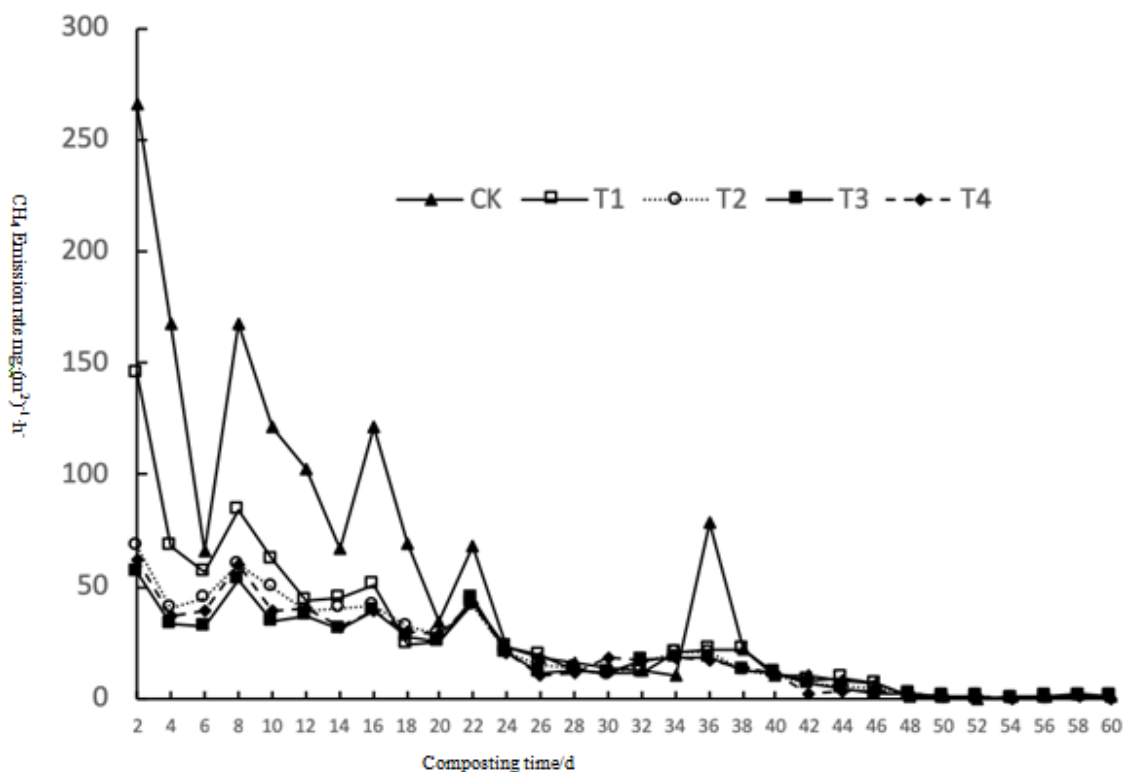

Figure 2. CH_4 emission rate during composting

The CH_4 emission peak was detected in CK treatment at the very beginning, where the mean emission rate was 49.52 mg/m²/h; the T1-T4 drainage rates were lower than that of CK, indicating that the addition of DCD and MHPP played a remarkable role in reducing emission from the composting of dairy manure and straws; measurement and calculation showed that the cumulative emission from CK was approx. 71.30 g/m², while the emission from T1-T4 was 39.56 g/m², 30.98 g/m², 27.15 g/m² and 28.81 g/m², which indicated the emission was reduced by 44.52%, 56.56%, 61.93% and 59.59%, respectively. It is observed that the effect of DCD to which calcium superphosphate was added was more obvious, while the addition of calcium superphosphate exhibited insignificant effect on MHPP emission reduction.

That the addition of calcium superphosphate and DCD help to reduce methane emission echoed the report of Luo et al.(2012), The main reason for such reduction is because that the sulfate ions in calcium superphosphate help to reduce the pile pH, which further inhibited the activity of methane bacteria, thereby realizing in the reduction of methane emission. In addition, since sulfate reducing bacteria reduce sulfate radicals into S^{2-} with acetic acid, lactic acid and hydrogen ions as electron

donors and with sulfate radicals as electron acceptors during composting, methane bacteria could finish their own biochemical metabolism; a competitive relation existed in between; additionally, S^{2-} had certain toxic effect on methane bacteria. However, the rate of emission reduction was not enhanced after the addition of calcium superphosphate to MHPP; the principal reason may be that the acidic calcium superphosphate brought about no promoter action in the ammonium nitrogen environment of MHPP.

The addition of calcium superphosphate reduced the carbon-nitrogen ratio, indicating that the addition of calcium superphosphate facilitated the ingestion of affecting component in microorganisms during composting, thus facilitating the microbial metabolism and the decomposition of organic matters.

Effect of the addition of MHPP on emission of N_2O as a greenhouse gas from compost

Ammonium nitrogen was continuously transformed into nitrate nitrogen during composting, and N_2O was frequently produced in this nitrification process; in addition, N_2O was generated during the denitrification from nitrate nitrogen to ammonium nitrogen. As can be seen from CK in *Figure 3,* the low temperature and adequate oxygen in early stage of traditional composting promoted the generation of abundant N_2O at compost surface, which was consistent with the studies of Hao et al. (2001) and Sommer et al. (2000). In the thorough decomposition period, the substantive production of nitrate nitrogen brought about denitrification that produced N_2O. The nitrates and nitrites generated in areas where there was plenty of oxygen were carried by pile turning into the anaerobic zone, where denitrification occurred while some N_2O was emitted, which was echoed the studies of Thompson et al. (2004) and Fukumoto et al. (2003); the nitrification process oxidized ammonia into hydroxylamine with the help of catalytic effect of ammonia monooxygenase on ammonia oxidizing bacteria and ammonia-oxidizing archaea; hydroxylamine produced NO_2^- under the action of hydroxylamine oxidoreductase, while NO_2^- was the key source of N_2O, so the major way to reduce N_2O should be the alleviation of nitration and denitrification reaction; there were two key ways of blockage: First, the production of initial NH_3 could be reduced by changing the pile pH; second, the ammonia monooxygenase or hydroxylamine reductase could be inhibited through DCD nitrification inhibitors.

In the present study, calcium superphosphate was used to improve the pile pH by reducing it below 6.5, thereby considerably reducing the emission of NH_3, the precursor of N_2O. As can be seen from *Figure 3,* the addition of DCD, calcium superphosphate and MHPP to T1-T4 caused the N_2O amount to drop significantly; the N_2O emission from T1, T2, T3 and T4 were 36.24%, 39.49%, 62.39% and 55.81% respectively less than the total N_2O emission from CK. The MHPP worked better in emission reduction; the effect of DCD with calcium superphosphate added was more obvious, while the effect of MHPP with calcium superphosphate added in emission reduction was inferior to the effect of MHPP without calcium superphosphate added.

Effect of MHPP addition on emission of harmful gas NH_3 from composting

As shown in *Figure 4,* a lot of ammonia was emitted in early stage of CK during traditional composting because the temperature rise in early stage of composting resulted in constant degradation of nitrogenous organic compounds, which produced

abundant ammonium nitrogen; in the stationary phase, the reduction of degradable nitrogen and the transformation of ammonium nitrogen into nitrate nitrogen or organic nitrogen enabled ammonia to effectively fix nitrogen in the form of ammonium phosphate when calcium superphosphate is added.
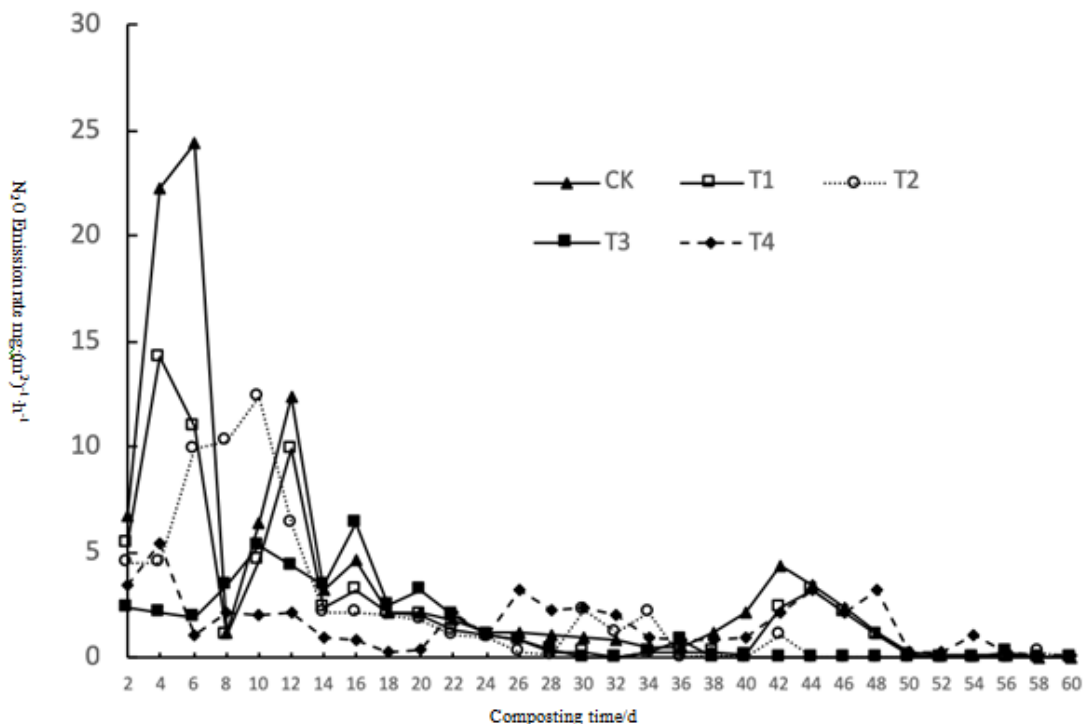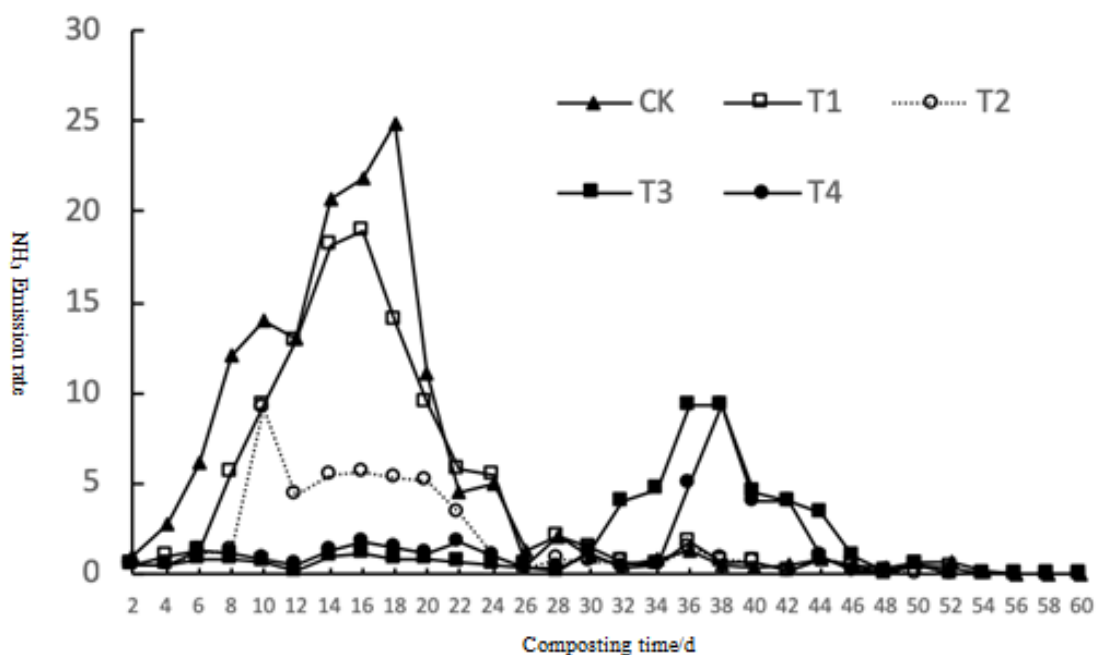

Figure 3. N_2O *emission rate during composting*

Figure 4. NH_3 *emission rate during composting*

The NH_3 emission during composting is shown in *Figure 4,* where the NH_3 emission of all the four treatments peaked during high-temperature period; nitrogenous organic compounds were substantively decomposed during high-temperature period, which produced a lot of ammonia. The ammonia emission was extremely low in the early and late stages of composting when temperatures were relatively low; throughout the stages, the emission of ammonia was reduced by T1-T4 by 23.72%, 65.61%, 65.40% and 72.61% respectively as compared with the control; the dominant mechanism was as follows: The acidic calcium superphosphate regulated the pH during composting below 6.5; as a result, N existed in the form of NH_4+, which went against the release of ammonia; in such a case, it existed in the form of ammonium acid phosphate or ammonium sulfate. The study performed by Li et al. (2008) demonstrated that the addition of calcium superphosphate reduced the ammonia emission from pig manure and rice straws by 39.39%; the value in the present study is greater than in that study, because nitrification inhibitor was added in the present study in addition to calcium superphosphate.

Variation of ammonium nitrogen (NH_4^+-N) content and nitrate nitrogen (NO_3^--N) contents

Ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) content variation is considered an important indicator for determination of nitrogen circulation during composting; refer to *Figure 5* for the variation pattern during composting; with the constant degradation of organic matters at early stage of composting, the composting of each treatment reached the high-temperature period, when microbial activity intensified, and the NH_4^+-N content of each treatment rose continually; it is observed that the NH_4^+-N from CK in traditional composting dropped rapidly after 28 days, while the NO_3^--N of CK shot up after the same period, which indicated that the NH_4^+-N of control was turning into NO_3^--N, and that the decrease was greater than the increase; and that the decrease was greater than the increase; this demonstrated that part of the nitrogen was emitted from the pile in the form of gas, namely NH_3 and N_2O. Where calcium superphosphate, DCD and MHPP were added to T1-T4, the NH_4^+-N of T1 slightly decreased in approx. 28 days, while that of T2, T3 and T4 did not went down significantly, which indicated the combination of calcium superphosphate and DCD alleviated the transformation and decomposition of NH_4^+-N; only the NO_3 -N of T1 slightly increased after 28 days while that of T2-T4 exhibited no significant rise, which demonstrated that calcium superphosphate, DCD and MHPP prevented NH_4^+-N turning into NO_3^--N, because DCD and MHPP inhibited the activity of nitrifying bacteria, thereby inhibiting or event preventing the nitrification in compost.

Physico-chemical and material decomposition indicators

Germination percentage is an important indicator for evaluation of compost decomposition; it is generally agreed that thorough decomposition is realized when GI > 80%; "thorough decomposition" means the application of fertilizer to crops bring about no secondary decomposition and lead to no adverse effects. In the present study, the germination percentage was higher than 96%, much higher than 80%; the reason why the germination percentage did not reach 100% may be because that it was impossible for any seeds to realize a germination percentage of 100%. A compost conductivity greater than 4 ms/cm may cause physiological damage to field crops, so

compost conductivity is also known as an important indicator of compost safety; as shown in *Table 3*, the conductivity dropped from 3.19–3.294 ms/cm to 0.96–1.89 ms/cm, which represented the compliance with safety requirements.
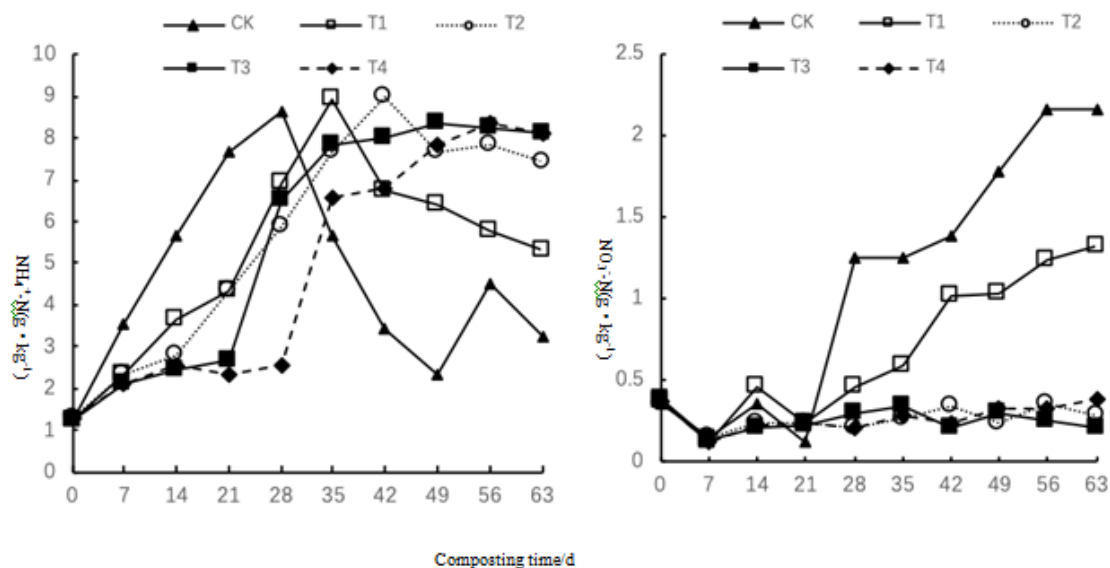

Figure 5. *Changes of NH_4^+-N and NO_3^--N content*

Table 3. *Chemical and physical characters and maturity index of composting*

Treatment	Compost Period	C/N	pH	Germination%	Electric conductivity EC/(ms·cm-1)
CK	Start	25.79a	7.85±0.08a	28.50±6.75a	3.25±0.15a
	End	10.23a	7.46±0.12b	97.75±0.25ab	0.96±0.08d
T1	Start	25.79a	7.81±0.03a	22.75±7.75d	3.29±0.21a
	End	10.09b	7.86±0.32a	98.75±0.50a	1.22±0.03c
T2	Start	25.79a	7.79±0.06a	23.25±3.50c	3.32±0.05a
	End	9.87c	7.16±0.09c	96.25±0.25b	1.68±0.08b
T3	Start	25.79a	7.19±0.03b	28.50±7.75a	3.23±0.04a
	End	10.13a	7.09±0.09d	98.75±0.75a	1.86±0.02a
T4	Start	25.79a	6.85±0.08c	25.50±5.75b	3.19±0.03a
	End	10.02b	7.12±0.09c	97.75±0.25ab	1.89±0.05a

Lower-case letters indicate significant differences at p < 5% level

Discussion

Chemically synthetic nitrification inhibitors principally include dicyandiamide (DCD), nitrapyrin and DMPP, which have been brought into production; however, these chemically synthetic inhibitors are not extensively used in agricultural production due to their high costs, their instable properties in different climates and soils (Li et al., 2008) and certain problems to be solved.

DCD, as a common nitrification inhibitor, can effectively inhibit the oxidation of ammonia, alleviate the leaching loss of nitrate nitrogen and the nitrogen loss resulting from denitrification, and improve the utilization of nitrogenous fertilizer (Moir et al.,

2010); however, there were some drawbacks with the application of DCD: The application rate of DCD had to reach 15–30 kg per Mu (666.7 square meters) so as to achieve inhibiting effect; DCD was extremely mobile in soil since it was easily soluble in water (Jacinthe et al., 1992), and the resulting spatial separation from ammonium nitrogen may impair the inhibiting effect; in the case of intense rainfall or soil with poor water retention capacity, leaching loss of DCD may frequently occur; the repeated application of DCD may cause the degradation rate to increase, thus reducing the half-time. Certain studies reported that the application of DCD may bring about toxic effects on crops (Xmb et al., 2003). The application of nitrapyrin increases the yield by 7%, reduces the nitrogen loss by 16%, and reduces the greenhouse gases emission by 51% (Woil et al., 2004); however, since nitrapyrin is a chlorinated organic matter, its long-term application may adversely affect the environment. Furthermore, nitrapyrin is prone to volatilization and photolysis, which makes it unsuitable for surface fertilization; moreover, the risk of corrosion and explosion makes it difficult to apply and keep this substance. Other studies showed that nitrapyrin exhibited certain toxicity that brought about curved and sallow leaves etc. in some plants (Maftoun et al.1981).

In contrast, the biological nitrification inhibitor MHPP produced through induction in ammonium nitrogen environment is a biological product produced by plants that are not harmful to the environment; since neither toxic nor side effect was detected during the test, MHPP is expected to be extensively generalized in composting and agricultural production. However, this substance is still under study, and the way to turn it into stable powder for extensive popularization is one of the important aspects of next step of research.

Conclusion

1. The combination of calcium superphosphate and MHPP did not cause the composting of straws and dairy manure to slow down, but improved the final compost quality; the ultimate germination percentage of each treatment was 97.5%, 98%, 98% and 97.5%, respectively.
2. The addition of calcium superphosphate and MHPP reduced the nitrogen oxide and ammonia emission during composting; the most appropriate content of MHPP was found to be 0.6%, where the emission of NH_3, N_2O and CH_4 were reduced by 65.40%, 62.39% and 61.93%, respectively.
3. Since the present test was a large engineering test featuring large mass of raw material and fermentation field, it was difficult to perform repeated tests; however, the gas sampling and chemical test indicates were repeatedly tested during the test after adequate mixing of raw materials, so the experimental data are reasonable and rational.
4. Recommendations: The compost will be used in field. We did not research how compost influence gas emission during plant growth. So further researches should be conduct on how compost influences gas emissions during using process.

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